There are many examples within gene complexes of transcriptional enhancers interacting only with a subset of target promoters. At the *Drosophila* bithorax complex (BX-C) over 300 kb of intergenic DNA sequence is responsible for directing expression of the three Hox genes during embryonic development. We have been examining the mechanisms which regulate promoter–enhancer interactions at the BX-C. In the BX-C, the IAB5 enhancer is located 55 kb 3′ of the Abdominal-B (Abd-B) promoter and 48 kb 5′ of the abdominal-A promoter. Although roughly equidistant from the two promoters, IAB5 specifically interacts only with the Abd-B promoter, even though the enhancer and promoter are separated by at least three insulators. Our experiments demonstrate that a novel 255 bp cis-regulatory module, the promoter tethering element (PTE), located 5′ of the Abd-B transcriptional start site is able to tether IAB5 to the Abd-B promoter in transgenic embryo assays. The PTE is sufficient to direct IAB5 to an ectopic promoter in competition assays. In order to analyze the in vivo function of the PTE, we studied flies in which the PTE had been disrupted by a P element insertion at the endogenous locus. Imprecise excision of the P element was used to generate a mutant featuring a partial deletion of the endogenous PTE sequence. Our analysis shows that disruption of PTE function results in a loss of Abd-B expression and is homozygous lethal in embryos.

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Program/Abstract # 158
Atypical protein kinase C and interferon regulatory factor 6 govern development of zebrafish superficial epithelium

Mallory Stroik[a], Katie Lutz[b], Jack R. Schleiffarth[c], Eric A. VanOtterloo[b], Elizabeth Leslie[b], Robert A. Cornell[b]
[a]*College of Letters, Arts, and Sciences, University of Iowa, Iowa City, IA USA*
[b]*Department of Anatomy and Cell Biology, College of Medicine, University of Iowa, Iowa City, IA USA*
[c]*Department of Otolaryngology, College of Medicine, University of Iowa, Iowa City, IA USA*

Mutations in the gene encoding transcription factor Interferon Regulatory Factor 6 (IRF6) confer risk for non-syndromic cleft lip and/or (CL/P), a common birth defect; however the regulatory pathway acting upstream of IRF6 is poorly defined. In zebrafish *irf6* mRNA is expressed in oral epithelium and the enveloping layer (EVL), a superficial epithelium (SE) which forms at blastula stage and becomes periderm. We have shown that inhibition of maternal Irf6 causes defects in differentiation EVL, leading to death during gastrulation. With a hormone-inducible form of dominant negative Irf6 we show here that inhibiting Irf6 after gastrulation disrupts skin development, similar to deletion of the *Irf6* gene in mice. Atypical protein kinase C (aPKC) activity is necessary for development of frog SE. We hypothesize that aPKC acts upstream of Irf6 during zebrafish EVL development, possibly by directly phosphorylating Irf6. Supporting this model, misexpression of an activated variant of aPKC gives rise to defects in EVL development. We show that exogenous Irf6 becomes phosphorylated in zebrafish embryos. To identify phosphorylated residues in Irf6, we are systematically substituting Thr/Ser residues, many of which lie within PKC consensus phosphorylation sites, with Alanines. Better knowledge of the Irf6 regulatory pathway may lead to improved ability to assess risk for CL/P.

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Program/Abstract # 159
Identification of alternative splicing in genes that determine sexual dimorphism in *Stiphra* sp (Orthoptera: Proscopiidea)

Mary Miller[a], Stephen Madigosky[a], Alexis Nagengast[b]
[a]*Department of Biology, Widener University, Chester, PA, USA*
[b]*Department of Biochemistry, Widener University, Chester, PA, USA*

The sex determination pathway in the fruit fly *Drosophila melanogaster* is regulated at the level of alternative splicing to produce male and female specific transcripts responsible for the morphological differences between the sexes. In *Drosophila*, Sexlethal (Sxl) activates the sex determination cascade while doublesex (dsx) performs the role of master regulator in other dipteran insects and silk worms. Despite these regulatory differences, sex specific alternative splicing appears to be a universal mechanism of sexual dimorphism among insects. We currently are culturing an unidentified species of jumping stick, *Stiphra* sp, from the Peruvian Amazon that exhibits dramatic sexual dimorphism and color variation. Adult females are cryptically colored in shades of brown and span approximately 17 cm from head to abdominal tip, while the males are green and much thinner, often measuring 10 cm in length. We hypothesize that the alternative splicing of homologous sex determination genes play a role in the morphological differences noted between the sexes of *Stiphra* sp. To investigate this, we are probing a *Stiphra* sp genomic library in an attempt to identify homologous sex determination genes. Additionally, we are using *Drosophila* microarray chips to analyze gene expression differences between the sexes in *Stiphra* sp. We expect to find male and female patterns of alternative splicing among conserved sex determination genes in *Stiphra* sp.

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Program/Abstract # 160
Role of the Trps1 transcription factor in odontoblasts differentiation and function

Dobrawa Napierala[a], Izabela Maciejewska[b], Elda Munivez[a], Brian Dawson[a,c], Rena D'Souza[b], Brendan Lee[a,c]
[a]*Baylor College of Medicine, Houston, TX, USA*
[b]*Baylor College of Dentistry, Dallas, TX, USA*
[c]*Howard Hughes Medical Institute, USA*

Trps1 is a transcription factor involved in bone development. Our previous studies demonstrated that during endochondral bone development Trps1 represses Runx2, which is a master regulator of osteoblast differentiation and chondrocyte maturation. Additionally, analyses of long bones of Trps1 mutant mice revealed advanced mineralization of the perichondrium in comparison with WT littermates, which suggests that Trps1 acts as a negative regulator of mineralization. To test this hypothesis we generated Trps1 transgenic mice over-expressing Trps1 in osteoblasts and odontoblasts (Col1a1–Trps1 mice). Col1a1–Trps1 mice demonstrate growth retardation after weaning and most die before 5 weeks of age due to feeding problems. Histological and micro-CT analyses reveal a dramatic decrease of dentin mineralization in transgenic mice. Additionally, expression of dentin sialoprotein (Dsp) and dentin matrix protein 1 (Dmp1), major non-collagenous proteins of dentin matrix, is reduced in Trps1 transgenic mice. During early tooth development, Trps1 is highly expressed in dental mesenchyme, and after establishment of the cell lineage, in pre-secretory odontoblasts and the dental sac. However Trps1 expression is diminished when odontoblasts mature and begin secreting matrix. The Trps1 expression pattern in teeth together with findings in Trps1 transgenic mice indicate that Trps1 expression must be turned off in secretory